(19) Europ[illegible]es Patentamt
European Pat nt Offic
Office europé n des br vets

(11) Publication number : **0 561 569 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number : **93301895.4**

(22) Date of filing : **12.03.93**

(51) Int. Cl.[5] : **C12N 15/82,** C12N 15/53, A01H 5/00, A01H 5/10, C11B 1/00, A23D 7/00

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(30) Priority : **13.03.92 US 850714**

(43) Date of publication of application : **22.09.93 Bulletin 93/38**

(84) Designated Contracting States : **AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE**

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(54) Modification of vegetable oils using desaturase.

(57) Plant seed having yeast delta-9 desaturase gene therein, preferably in association with a suitable promoter and termination sequence. A method for modifying the fatty acid content of seed oil, by transforming seed with yeast delta-9 desaturase.

EP 0 561 569 A2

1. Field of the Invention

The present invention relates to a plant seed containing the yeast delta-9 desaturase gene, and to a method for modifying the fatty acid content of seed oil using the desaturase gene.

More specifically, the present invention is directed to a plant seed, such as a Brassica or Zea mays seed, containing the yeast delta-9 desaturase gene under the control of a promoter which causes expression of the gene in the seed and, as a corollary, to a method of modifying the seed oil fatty acid content using the desaturase gene.

2. Description of Background and Relevant Information

Vegetable oils are used not only in the food industry, but increasingly in the chemical industry as well, and are starting to find their way into industrial applications as alternatives to more conventional lubricating fluids. The utilization of the oils depends principally on their compositions. Triglycerides comprise the bulk of vegetable oil (about 95%), but a number of other important lipids are also present, such as phospholipids, free fatty acids, waxes, and sterols. A variety of other components, such as anti-oxidants, may also be present which, while occurring in relatively minor amounts, may nonetheless have a significant impact on the characteristics and, hence, utility of the oils.

The characteristics of triglycerides depend in large measure upon their constituent fatty acids. Because the fatty acids which occur naturally in agronomically acceptable strains of seed oil crops frequently render the resulting oil unsuitable for an otherwise attractive use, it is extremely desirable to have the ability to change the oil composition to meet specified parameters.

Modification of vegetable oils may be effected chemically. This approach has been used to obtain a salad/cooking oil which contains saturated fatty acids of less than about 3% (United States Patent Number 4,948,811); the oil may be formed by chemical reaction, or by physical separation of the saturated lipids. A general reference is made to using "genetic engineering" to achieve an oil of the desired characteristics (see column 3, line 58 et seq.). However, there is no detailed disclosure of how any particular oilseed plant could be so modified to provide a vegetable oil of the characteristics desired.

Typically, the fatty acid composition of vegetable oils has instead been modified through traditional breeding techniques. These techniques utilize existing germplasm as a source of naturally-ocurring mutations which affect fatty acid composition. Such mutations are uncovered and selected for by the use of appropriate screening, in conjunction with subsequent breeding. For example, such an approach has been used to decrease the amount of the long chain fatty acid erucate in rapeseed oil (Stefansson, B.R. (1983) in High and Low Erucic Acid Rapeseed Oils, Kramer JKG et al., eds; Academic Press, NY; pp. 144-161), and to increase the amount of the monounsaturated fatty acid oleate in corn oil (United States Patent Application, Serial Number 07/554,526).

Recently, attempts have been made to increase the pool of available mutations from which to select desired characteristics through the use of mutagens. However, mutagens generally act by inactivation or modification of genes already present, resulting in the loss or decrease of a particular function. The introduction of a new characteristic through mutagenesis thus often depends on the loss of some trait already present. In addition, the achievement of desired goals with mutagens is generally uncertain. Only a few types of modified fatty acid compositions in vegetable oils have been achieved using this approach. One example of such a "created" mutation which affects fatty acid composition is the decrease of polyunsaturated fatty acids, in particular of linoleate and linolenate, in rapeseed oil, with a concommittant increase in the monounsaturated fatty acid oleate (Auld, M., et al., (1992) Crop Sci. in press). Another is the decrease of saturated fatty acids in rapeseed oil (PCT International Patent Application Publication Number WO 91/15578). However, the biochemistry of seed oil synthesis is complex, and not well understood; there may be several mechanisms which contribute to the changes in the fatty acid compositions observed in rapeseed oil (PCT International Patent Application Publication Number WO 91/ 15578). The use of mutagenesis to affect such changes is essentially random, and non-specific.

The possibility of modifying fatty acid composition through the use of genetic engineering would, in theory, allow the precise, controlled introduction of specific desirable genes, as well as the inactivation of specific undesirable genes or gene products. Thus, novel traits completely independent of genes already present could be introduced into plants, or pre-selected genes could be inactivated or modified. However, one predicate to making effective use of genetic engineering to modify fatty acid compositions is a reasonably accurate model of the mechanisms at work in the plant cell regulating fatty acid synthesis and processing.

It is postulated that, in oilseeds, fatty acid synthesis occurs in the plastid, and that the newly synthesized fatty acids are exported from the plastid to the cytoplasm. Here they are utilized in the assembly of triglycerides,

which occurs in the endoreticular membranes.

The major product of fatty acid synthesis is palmitate (16:0), which appears to be efficiently elongated to stearate (18:0). While still in the plastid, the saturated fatty acids may then be desaturated, by an enzyme known as delta-9 desaturase, to introduce one or more carbon-carbon double bonds. Specifically, stearate may be rapidly desaturated by a plastidial delta-9 desaturase nzyme to yield oleat (18:1). In fact, palmitate may also be desaturated to palmitoleate (16:1) by the plastidial delta-9 desaturase, but this fatty acid appears in only trace quantities (0 - 0.2%) in most vegetable oils.

Thus, the major products of fatty acid synthesis in the plastid are palmitate, stearate, and oleate. In most oils, oleate is the major fatty acid synthesised, as the saturated fatty acids are present in much lower proportions.

Subsequent desaturation of plant fatty acids outside the plastid in the cytoplasm appears to be limited to oleate, which may be desaturated to linoleate (18:2) and linolenate (18:3). In addition, depending on the plant, oleate may be further modified by elongation (to 20:1, 22:1, and/or 24:1), or by the addition of functional groups. These fatty acids, along with the saturated fatty acids palmitate and stearate, may then be assembled into triglycerides.

The plant delta-9 desaturase enzyme is soluble. It is located in the plastid stroma, and uses newly-synthesized fatty acids esterified to ACP, predominantly stearyl-ACP, as substrates. This is in contrast to the yeast delta-9 desaturasé enzyme, which is located in the endoplamsic reticular membrane, uses fatty acids esterified to Co-A as substrates, and desaturates both the saturated fatty acids palmitate and stearate.

The yeast delta-9 desaturase gene has been isolated from Saccharomyces cerevisiae, cloned, and sequenced (Stukey, J.E. et al., J. Biol. Chem. **264** :16537-16544 (1989); Stukey, J.E. et al., J. Biol. Chem. **265**:20144-20149 (1990)). This gene has also been used to transform the same yeast strain under conditions in which it is apparently overexpressed, resulting in increased storage lipid accumulation in the transformed yeast cells as determined by fluorescence microscopy using Nile Red as a stain for triglycerides (U.S. Patent Number 5,057,419). The fatty acid composition was not characterized. This reference contains a general discussion of using information from the isolated yeast delta-9 desaturase gene to first isolate other desaturase genes from yeast, or from other organisms, and then to re-introduce these genes into a yeast or plant under conditions which, it is speculated, could lead to high expression, in order to modify the oil produced and its fatty acid composition (see Example 2, at column 9, lines 24 et seq.). However, this discussion is both general and hypothetical. No actual examples are provided, and the only technique offered for accomplishing this goal is a recitation of classic recombinant DNA methodology without guidance as to specific implementation (see column 10, lines 25 et seq.).

Subsequently, it was reported that the yeast delta-9 desaturase gene had in fact been introduced into tobacco leaf tissue (Polashcok, J. et al., FASEB J. 5:A1157 (1991). Apparently, the gene was expressed in this tissue, as evidenced by a reported ten fold increase in palmitoleic acid and a corresponding decrease in palmitic and stearic acids.

The health value of high levels of monounsaturates, particularly oleic acid, as the major dietary fat constituent has been established by recent studies. Such diets are thought to reduce the incidence of arteriosclerosis that results from diets high in saturated fatty acids. There is accordingly a need for an edible vegetable oil having a high content of monounsaturates. Seed mutagenesis has been used to produce a rapeseed oil with no more than 4 % saturated fatty acid content (PCT International Patent Application Publication Number WO 91/15578); the lowest value reported was a single seed value of 2.8% saturated fatty acid content. However, this low saturated fatty acid vegetable oil is limited to rapeseed oil.

Expression of the yeast delta-9 desaturase gene in any plant seed tissue could result in a decrease in the saturated fatty acids, with an increase in monounsaturated fatty acids in the seed oil. In this case, the enzyme is proposed to desaturate those saturated fatty acid which are exported from the plastid and thus no longer a substrate for fatty acid desaturation. Thus, transformation of plants with a yeast delta-9 desaturase gene under conditions in which the gene is expressed in the seed tissue leads to decreased saturated fatty acid seed oil.

In addition, expression of the yeast desaturase gene in plants with unusual fatty acid compositions could result in the increase or appearance of unusual fatty acids is the vegetable oil. For example, expression of the yease delta-9 desaturase gene in seed tissue in which the oil contains high levels of palmitate could result in an increase in the level of palmitoleate. In those tissues in which fatty acid elongation occurs (such as a high erucate rapeseed), longer chain fatty acids with unusual doubl bonds could accumulate. Such fatty acids include cis-vaccenic (18:1 cis 11), 20:1 cis 13, 22:1 cis 15, and 24:1 cis 17. These fatty acids are of industrial interest. For example, oxidative ozonolysis cleavage of 18:1 cis 11 results in the monobasic C7 fatty acid and th dibasic C10 fatty acid. Both th dibasic and monobasic fatty acids are an industrial raw material and commodity fatty acids. They,..can be us d as replacements, or in situations where a specific functionality is desired.

SUMMARY OF THE INVENTION

There is accordingly provided a plant seed comprising a yeast delta-9 desaturase gene and means for expressing the y ast delta-9 desaturase gene in the plant seed. The means for expressing may comprise a promoter effective to cause expression of the yeast delta-9 desaturase g ne in the plant seed, and the promoter may be, for example, a phaseolin promoter, a truncated phaseolin promoter, and a 35S promoter. Preferable the promoter is a seed-specific promoter, and most preferably it is truncated phaseolin promoter.

The plant seed may also contain a termination sequence for the yeast delta-9 desaturase gene, such as a yeast delta-9 desaturase termination sequence, a phaseolin 3' termination sequence, or a ORF 25 3' termination sequence.

The plant seed may be a member of a monocot genus, including Zea and Sorghum, with Zea, and particularly Zea maize, being preferred. Alternatively the plant seed may belong to a dicot genus, such as Brassica, Helianthus, Carthamus, Sesamum, Glycine, Arachis, Gossypium, Lesquerella, and Vernonia, in which case Brassica, and particularly Brassica rapa and Brassica napa, are preferred.

In a further embodiment, the present invention is directed to a method for modifying the fatty acid content of the seed oil of a plant seed by transforming the plant seed to express a yeast delta-9 desaturase gene. The modification may involve increasing the percent content of monounsaturated fatty acid in the seed oil of the plant seed. The monounsaturated fatty acid so affected may have a carbon chain length of from 16 to 24 carbon atoms, such as, for example, cis-9-hexadecanoic acid (palmitoleic acid), cis-9-octadecanoic acid (oleic acid), cis-11-octadecenoic acid (cis-vaccenic acid), cis-11-eicosenoic acid, cis-13-eicosenoic acid, cis-13-docosenoic acid, cis-15-docosenoic acid, cis-15-tetracosenoic acid, cis-17-tetracosenoic acid, and combinations thereof. The monounsaturated fatty acid may also be oleic, palmitoleic, or cis vaccenic acid.

Alternatively, the fatty acid content may be modified by reducing the percent content of saturated fatty acid in the seed oil. The saturated fatty acid may be myristic acid, palmitic acid, stearic acid, eicosanoic acid, docosanoic acid, tetracosanoic acid, and combinations thereof.

In this method, the plat seed to be modified may be selected from the same monocot and dicot genera and species listed above.

The transformation is generally accomplished by adding, to the native DNA of the plant seed, exogenous DNA in the form of yeast delta-9 desaturase gene and a promoter for the yeast delta-9 desaturase gene. Suitable transformation techniques include transformation mediation using Agrobacterium, electroporation, polyethylene glycol (PEG), silicon carbide fiber, particle gun, and direct injection.

In a particular embodiment, the transformation contemplates constructing a vector containing the yeast delta-9 desaturase gene and the promoter, placing the vector into a selected strain of Agrobacterium, and treating selected plant cells with the Agrobacterium under conditions sufficient to result in transfer of at least some of the vectors from the Agrobacterium to the plant cells, whereby the yeast delta-9 desaturase gene is expressed in the plant cells.

In a final embodiment, the present invention is directed to a plant obtained from the plant seed as defined by claim 1, wherein the seeds of the plant comprise a yeast delta-9 desaturase gene and means for expressing the yeast delta-9 desaturase gene in the plant seed.

3. Brief descriptions of the drawings

Figure 1 shows the DNA sequence and partial restriction map of the yeast delta-9 desaturase gene. The coding strand of DNA along with the deduced amino acid sequence of the desaturase gene is shown. These sequences are also represented in ID SEQ Nos. 1 and 2 appendexed hereto.

Figure 2 depicts a scheme of plasmid pH602 and four plant expression cassettes with the yeast delta-9 desaturase gene which were cloned into the unique BglII site of the plasmid.

4. Detailed Description of the Preferred Embodiments

In Brief. By means of the present invention, there is provided a plant seed containing and expressing a yeast delta-9 desaturase gene. In addition, there is provided a method for obtaining vegetable oil having an altered fatty acid profile by transforming a plant with a yeast delta-9 desaturase gene under conditions in which the yeast delta-9 gene is expressed in the seed.

In summary form, a yeast delta-9 desaturase gene can be isolated by the following steps. Th gene is first cloned by in vivo complementation of a yeast ole1 mutant strain to OLE+ by transformation with a yeast genomic bank made from wild-typ yeast DNA. Plasmid-bome complementing sequences can be characterized by restriction mapping, verified by genetic means, and sequenced.

The coding sequence of the gene is placed under the control of regulatory sequences which function in a plant se d, and is then mov d into plant transformation vectors. These constructs, with a suitabl selectable marker to select for positive transformants, are then used to transform plant tissue. The resulting calli are regenerated into plants; tissue samples from these plants are screened by at least one molecular or biological assay to determine which individuals actually contain a yeast delta-9 desaturase gen .

Those transformants which contain the yeast delta-9 desaturase are grown to maturity and allowed to set seed. The expression of the yeast delta-9 desaturase gene in the seed is determined by mRNA analysis, such as with a PCR assay, and/or by protein analysis, such as by a Western assay. In addition, the fatty acid composition of the mature seeds is determined to identify any novel fatty acid composition produced in response to the presence of the yeast delta-9 desaturase in the seeds. Those seeds showing altered fatty acid composition are germinated, and the stability and genetics of the observed trait(s) characterized by the proper genetic crosses.

Each of the aspects of the invention will now be discussed in greater detail.

The majority of the DNA and yeast genetic manipulations described below are standard, well-established protocols and can be found in several protocol manuals (Sherman, F et al (1986) Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, NY; Sambrook, J et al (1989) Molecular Cloning, A laboratory Manual, Cold Spring Harbor Laboratory Press, NY; and Methods in Enzym 152: Guide to Molecular Cloning Techniques, Berger, SL and Kimmel, AR, eds (1987). DNA can be sequenced by several methods, including for example the Sanger method, using the protocol for double-stranded templates as per the manufacturer's (U.S. Biochem. Corp., Cleveland, OH) instructions.

A delta-9 desaturase gene from yeast can be isolated by the following steps. A yeast strain is created which is suitable for transformation and complementation. Such a strain must be deficient in delta-9 desaturase activity and thus require exogenous unsaturated fatty acids for growth. In addition, the strain may have a second characteristic or marker to allow for the selection of transformed cells. A genomic bank of wild type yeast DNA is then be used to transform the yeast strain deficient in delta-9 desaturase activity. Those strains in which restoration of the delta-9 desaturase function was observed (ie, which exhibited a wild-type phenotype and no longer required exogenous fatty acids for growth) are presumed to contain the yeast delta-9 desaturase gene. The recombinant plasmids inserts which putatively contain the delta-9 desaturase gene are then isolated. A restriction map of the insert is prepared, and can be compared to a published map (Stukey, JE (1989) J. Biol. Chem. 264: 16537-16544). Finally, the identity of the gene is verified by standard genetic analysis.

The insert DNA is then subcloned and sequenced to locate and characterize the coding region of the yeast delta-9 desaturase gene. The coding region is moved into plant expression cassettes after selecting the proner regulatory sequence(s) for desired expression. Regulatory sequences include both promoter and termination sequences.

Possible regulatory sequences include, but are not limited to, any promoter already shown to be constitutive for expression, such as those of viral origin (CaMV 19S, TMV, AMV) or so-called "housekeeping" genes (ubiquiton, actin, tubulin) with their corresponding termination/polyA+ sequences. Also, seed and/or developmentally specific promoters, such as those from plant fatty acid/lipid biosynthesis genes (ACPs, acyltransferases, desaturases, lipid transfer protein genes) or from storage protein genes (zein, napin, cruciferin, conglycinin, lectin genes), with their corresponding termination/polyA+ sequences can be used for targeted expression. In addition, the gene can be placed under the regulation of inducible promoters and their termination sequences so that gene expression is induced by light (rbcS-3A, cab-1), heat (hsp gene promoters) or wounding (mannopine, HRPGs). It is clear to one skilled in the art that a promoter may be used either in native or truncated form, and may be paired with its own or a heterologous termination/polyA+ sequence.

In addition, the yeast delta-9 desaturase gene product may be localized to a specific organelle in the plant seed by ligating DNA coding for peptide leader sequences to the desaturase gene. Such leader sequences are obtained from several genes of either plant or other sources. These genes encode cytoplasmically-synthesized proteins directed to, for example, mitochondria (the F1-ATPase beta subunit from yeast or tobacco, cytochrome c1 from yeast), chloroplasts (cytochrome oxidase subunit Va from yeast, small subunit of rubisco from pea), endoplasmic reticulum lumen (protein disulfide isomerase), vacuole (carboxypeptidase Y and proteinase A from yeast, phytohemagglutinin from French bean), peroxisomes (D-aminoacid oxidase, uricase) and lysosomes (hydrolases). These constructs may be used with the corresponding native promoter or with any of the suggested promoters mentioned above.

A selectable marker for optimum transformation selection is also chosen. Such markers are typically genes which encode for resistance to various toxic chemicals such as antibiotics and herbicides; the resistance is usually conferred by enzymes which typically rend r the chemical non-toxic. Such toxic chemicals include, for exampl , hygromycin, kanamycin, m thotrexate, and phosphinothricin. Enzymes which confer resistanc to these chemicals are hygromycin phosphotransf rase, neomycin phosphotransferase, dihydrofolat reductase,

and phosphinthricin acetyl transferase. Genes which code for resistance are well known to those of ordinary skill in the art of plant transformation. Plants transformed with such genes are able to grow in the presence of the toxic compound, while non-transformed plants are not. Therefore, such g nes serve both as a means of selecting transformed plants and as a marker for transformation, indicating that transformation has occurred.

Finally, th plant xpression cassette containing the yeast delta-9 desaturas gene is moved into the vector which also contains the selectable marker for use in plant transformation. The selectable marker is typically under the control of a constitutive promoter as are described above. The vector is constructed in such a manner that both the yeast delta-desaturase gene and the marker gene are transferred together into the plant genome.

Plant tissue for use in transformation may be obtained from any suitable oilseed plant. Such plants may be found in the genera Brassica, Helianthus, Carthamus, Sesamum, Glycine, Arachis, Gossypium, Ricinus, Linum, Cuphea, Euphorbia, Limnanthes, Crambe, Lesquerella, Vernonia, Simmondsia, Olea, Papaver, Elaeis, Cocos, and Zea. Appropriate plant tissue includes but is not limited to leaves, hypocotyls, colyledons, stems, callus, single cells, and protoplasts.

Transformation techniques are well known to those skilled in the art of plant transformation, and include transformation mediated by Agrobacteria, electroporation, polyethylene glycol (PEG), silicon carbide fibers, direct injection and a particle gun. These methods are various means to introduce foreign DNA into plant cells. Once in the cell, a portion of the DNA carrying both the yeast delta-9 desaturase gene and the selectable marker are incorporated into the plant genome via the transfer functions included in the DNA.

Transformed callus tissue is selected by growth on selection medium (eg, medium which contains a toxic chemical and for which the transformed plant contains a resistance gene, by virtue of its transformation). Transformed plants are regenerated and screened for the presence of the yeast delta-9 desaturase gene. This involves analyzing tissue by at least one molecular or biological assays to determine which, if any, transformants contained yeast delta-9 desaturase specific mRNA or DNA sequences. These assays include assays of the tissue for the expression of the resistance gene enzyme, and assays of the tissue for the presence of the yeast delta-9 desaturase DNA by for example, a Southern assay or a PCR assay.

Those plants which are positive for the yeast delta-9 desaturase gene are grown to maturity, pollinated, and allowed to set seed. Seed obtained from transformed plants are analyzed for the expression of the yeast delta-9 desaturase gene by both looking for the protein encoded by the gene, as for example via a Western analysis, and for the phenotype of altered fatty composition as a result of the activity of the desaturase.

A Western analysis determines the presence of a protein encoded and expressed by the yeast delta-9 desaturase gene, and is utilized to detect expression of the gene in plant seed tissue. The assay requires the use of antibodies to the yeast delta-9 desaturase to detect the presence of the protein. Antibodies specific for the yeast delta-9 desaturase protein, which has not been previously purified, are prepared as follows. The coding sequence for the yeast delta-9 desaturase enzyme is cloned into an expression vector (for example, pMAL-p, pMAL-cRI, from New England Biolabs). The resulting protein is isolated and purified according to the manufacturers instructions. Antibodies are then generated to the delta-9 desaturase enzyme by conventional techniques. The specificity of the antibodies to the delta-9 desaturase enzyme is determined by ELISA assays.

The fatty acid composition of either whole or half-seeds, obtained from either control or transgenic plants, are determined by extracting the oil, preparing fatty acid methyl esters, and then separating and quantitating the fatty acid methyl esters by conventional procedures. Novel fatty acid characteristics are determined by comparing the fatty composition of the transgenic seeds to those of the parent plant.

The genetic stability and inheritance of the novel fatty acids traits are determined by classic genetic crosses. The trait(s) conferred by the yeast delta-9 desaturase gene may be transferred into other agronomically acceptable cultivars by standard breeding technology.

EXAMPLE 1

Gene Cloning, Isolation, and Sequencing

a. Gene Cloning and Isolation

The delta-9 desaturase gene was cloned in yeast by complementation using a 2-micron vector and looking for cosegregation of markers. A genomic bank of wild type yeast DNA from Saccharomyces cerevisiae strain X2180-1A (the Yeast Genetic Stock Center, Berkeley, CA) was prepared in the yeast autonomous plasmid, YEp13. This plasmid transforms at a high efficiency and replicat s independ ntly of the chromosomes to a high copy number. YEp13 contains a wild type LEU2 gene for selection of transformants and is mitotically unstable when grown und r nonselective conditions. Upon transformation into E. coli, the bank gav 1.7x104 independent transformants, well above the 9.5x103 figure calculated as necessary to result in a 99% probability

that a copy of the y ast delta-9 desaturase gene was contained in the bank.

A yeast strain suitable for transformation was constructed by mating two haploid yeast strains and analyzing the resultant haploid spores. One strain was found to contain both mutations (ole1/leu2) needed for complementation. A yeast transformation was performed and transformants selected for both the LEU2. marker contained on the plasmid and for wild type desaturase activity by growth on medium lacking ol ate. In this way, 450 transformants which were phenotypically wild type for both LEU2 and desaturase activity were obtained. These transformants were next grown under nonselective conditions, thereby inducing mitotic instability, and then screened for co-loss of the LEU2 plasmid marker and desaturase activity. Co-loss of markers indicated that the desaturase activity was plasmid-bome and not a chromosomal revertant to wild type.

In this way, several plasmid-bome inserts that restored wild-type function when present in mutant yeast cells were identified. A 5.7kb Hind III fragment was found to be common to most of the inserts, and restriction map analysis of the insert indicated extensive homology to that of the published restriction map (Stukey, JE et al (1989) J. Biol. Chem. 264: 16537-16544). The major difference between the two maps is that the published insert is a 4.8kb Hind III fragment, compared to the 5.7kb Hind III fragment isolated here. However, the region has been shown to be polymorphic in various yeast strains, including the progenitor strain of the strain from which the genomic bank was made (Stukey, JE et al, supra). Additionally, the restriction map is highly conserved in the putative yeast delta-9 desaturase coding region in the insert; the difference in size is due to downstream sequences outside the coding region.

An attempt was made to identify, by Southern analysis, plant DNA sequences that showed homology to the yeast desaturase gene. Genomic DNA from Brassica napus, Brassica rapa, Arabidopsis thaliana, soybean, and maize was digested with each of three restriction enzymes and blotted to a membrane; the membrane was then probed with two random-primer labeled subcloned inserts which contain only open reading frame sequences from the yeast delta-9 desaturase gene. After hybridization, the filter was washed sequentially under increasingly stringent conditions, in order to determine if the signal-to-noise ratio of any homology could be reduced enough to make screening a genomic bank using the same probe feasible. Using the least stringent wash conditions, faint bands could been seen for all the genomes, but, not unexpectedly, heavy background was observed. Under the most stringent (i.e., "normal") wash conditions, the background was noticeably reduced; however, under these conditions, only the maize and the soybean DNA showed obvious bands which were still very faint, especially when compared to those in the yeast DNA.

These data indicate that the yeast delta-9 desaturase gene has little homology to its corresponding gene in Brassica, at least at the DNA level. Because the yeast delta-9 desaturase has both a different location (microsomal vs. chloroplastic) and substrate specificity (fatty acyl-CoA thioesters vs. fatty acyl-ACP thioesters) than does the plant enzyme, it is not unexpected that the two enzymes, despite their similar function, may show little homology at either the DNA or protein level.

Gene disruption studies corroborated the complementation results which indicated that the cloned gene was in fact the yeast delta-9 desaturase gene. A construct was made which inserted a functional LEU2 gene into the coding region of the putative yeast delta-9 desaturase gene, thereby disrupting the cloned gene. Using standard yeast genetic techniques, the chromosomal copy of the cloned gene was replaced with the LEU2-disrupted version and the resulting cells were then analyzed for their ability to grow without supplemental oleic acid. In this analysis, if the cloned gene was not the yeast delta-9 desaturase, then its disruption shouldn't affect fatty acid biosynthesis. If the cloned gene was the yeast delta-9 desaturase, then its disruption should result in cells that cannot make oleic acid and therefore require a supplemental source. In fact, yeast cells verified by Southern analysis to contain the disrupted gene cannot grow without supplemental oleic acid.

b. Gene Sequencing

Restriction fragments of the yeast delta-9 desaturase gene produced by digests with several different enzymes were subcloned into the vector pUC18. These fragments were sequenced using double stranded templates with Sequenase (USB) acccording to the manufacturer's instructions. The results are presented in Figure 1.

DNA sequence analysis of the cloned insert identified an open reading frame of 1530bp with a TATAA sequence, the preferred promoter sequence in yeast, located at -30 from the first ATG of the predicted protein which is generally used as the initiation codon of a peptide in yeast. Northern analysis demonstrated that DNA sequenc s from the open reading frame hybridized to a polyadenylated mRNA of approximat ly 2.0kb. Th transcript size observed is a good fit for a 1530bp coding sequence, allowing adequate room for a 3' polyA + tail, and sugg sts that the transcript, like most yeast mRNAs, contains no introns.

The 1530bp coding region of the cloned gene, assuming the translational start is the first ATG in the reading frame after the TATAA box, cod s for a prot in of 510aa. Homology search s of known protein data banks

hav shown that the predict d amino acid sequence of the yeast delta-9 desaturase gene is homologous to both the rat and mouse stearyl-CoA desaturase prot ins. Interestingly, homology to both mammalian proteins starts at around 42aa and nds at 397aa. Although the sequence of the clon d yeast delta-nine desaturase gene isolat d from Saccharomyces cerevisiae strain X2180-1A is very similar in sequence to that r ported isolated from the strain R 254 (originally designated AB320) (Stukey, JE et al (1990) J. Biol. Chem. 265: 20144-20149), th re is at least one amino acid difference between the two coding regions: th published sequenc reports a met at amino acid position 304, as opposed to a leu at the same position observ d here.

The data obtained from gene complementation studies, gene disruption studies,and sequence analysis have demonstrated that the cloned gene was the yeast delta-nine desaturase.

EXAMPLE 2

Vector Construction

Four expression vectors were constructed, in which the yeast delta-nine desaturase gene was placed under the control of different promoters and followed by different termination/poly-adenylation sequences. The vector used for plant transformation contained both the desired selectable marker and the yeast delta-9 desaturase gene expression cassette.

The tansformation vector into which the yeast delta-9 desaturase gene expression cassettes were placed is pH602 (see Figure 2; REF). This vector is a micro Ti plasmid binary vector similar to plasmid pH575 described previously (Hoffman, LM et al (1987) EMBO J. 6: 3213-3221) except that it contains as a selectable marker the hygromycin phosphotransferase (HPT) gene instead of a neophosphotransferase II (NPTII) gene (Murray, EE et al (1991) Plant Mol. Biol. Reporter 16: 1035-1050). The HPT gene, which confers resistance to the antibiotic hygromycin, is under control of the constitutive promoter CaMV 35S.

In order to obtain expression of the yeast delta-9 desaturase gene, the gene was put under the control of a seed-specific phaseolin promoter, obtained from the French bean, Phaseolus vulgaris (REF). Because seed oil accumulation occurs earlier than does the accumulation of seed storage protein during seed development and maturation, the phaseolin promoter is not optimal in terms of temporal regulation. Therefore, the gene was put under the control of a modified phaseolin promoter designed to be expressed earlier than the native phaseolin (Bustos et al (1991) EMBO J. 10: 1469-1479), as well as under the control of the constitutively expressed CaMV 35S promoter.

a. pH.PO

In this vector, the yeast desaturase gene was placed under the control of a seed specific promoter, the seed storage protein phaseolin promoter, and was followed by the yeast desaturase termination sequences.

The vector pSPPneo contained a genomic phaseolin gene and was the source of the promoter used in this construct. The vector was digested with EcoR1 and Sca1; the resulting 1.4 kb EcoRI-Sca1 fragment which contained the phaseolin promoter along with a multiple cloning site was isolated and cloned into pUC18, resulting in the vector designated scp5'phas.

The next step removed the multiple cloning site from the phaseolin promoter. scp5'phas was digested with EcoRI and EcoRV, both ends were filled in, and the vector religated. The resulting vector was designated scp-5'phas-delta, and contained the phaseolin promoter region minus the multiple cloning site.

A 5.8 kb HindIII fragment of yeast genomic DNA that contained the yeast desaturase gene (see above) was isolated and cloned into the HindIII site of pUC18, resulting in the vector designated pUC26A. A 3.5 kb SnaB1-Xho1 fragment which contained the entire yeast desaturase coding sequence and termination sequence was then isolated from pUC26A and cloned into the Sma1-Sal1 site of scp5'phas-delta. The resulting vector was designated pPO.

Finally, a 4.5 kb Nhe1-HindIII fragment, which contained the phaseolin promoter and the yeast desaturase coding and termination sequences, was isolated from pPO; this fragment was filled in, BamHI linkers were added, and the fragment was cloned into the BglII site of pH602. The resulting vector was designated pH.PO.

b. pH.POP

In this v ctor, the y ast desaturas gene was placed under control of the phaseolin promoter and followed by th phas olin termination sequenc s.

A 2.6 kb Nh 1-BspH1 fragm nt from pPO (see above), which confined the phaseolin promoter and th y ast desaturase coding sequ nce, was isolated. The BspH1 site was filled in, Pst1 link rs were added and

the fragment was cloned into the Xba1-Pst1 sites of pUC18. The resulting vector was designated pPO-2.

A 1.5 kb Pst1-Sst1 fragment from pSPPneo, which contained the phaseolin 3' terminating sequences, was isolated. The Sst1 site was filled in, Pst1 linkers were added, and the fragment was cloned into the PstI site of pPO-2. The resulting vector was designated pPOP.

pPOP was digested with BamHI, and the insert which contained the phaseolin promoter, the yeast desaturase gene, and the phaseolin 3' termination sequence was cloned into the BglII site in pH602. The resulting vector was designated pH.POP.

c. pH.PdeltaBOP

In this vector, the yeast desaturase gene was placed under control of a modified phaseolin promoter and was followed by the phaseolin termination sequences. The phaseolin promoter was modified by truncation, which has been reported to result in earlier expression of genes regulated by the promoter (Bustos et al (1991) The EMBO J 10: 1469 - 1479).

A 2.0 kb Bcl1-Pst1 fragment from pPO-2 (see above), which contained a truncated phaseolin promoter and the yeast desaturase coding sequence, was isolated. The truncated phaseolin promoter contained only about a third, or 295 bp, of the original promoter sequence. This fragment was cloned into the BamH1-Pst1 sites of pUC18. The resulting vector was designated pPOdeltaB.

A 1.5 kb Pst1 fragment, which contained the 3' polyadenylation sequence of the phaseolin gene, was isolated from POP (see above). This fragment was inserted into the PstI site of pPOdeltaB, to create a construct containing a truncated phaseolin promoter, the yeast desaturase gene, and the phaseolin termination sequence. The resulting vector was designated pPdeltaBOP.

A 3.2 kb BamH1 fragment from pPdeltaBOP, which contained the truncated phaseolin promoter, the yeast desaturase coding sequence, and the phaseolin termination sequence, was isolated. This fragment was inserted into the BglII cloning site of pH602. The resulting vector was designated pH.PdeltaBOP.

d.pH.SOA

In this vector, the yeast desaturase gene was placed under control of a constitutive promoter, the 35S promoter, and was followed by the termination sequences from ORF25.

The vector pIC35/A contains the CaMV 35S promoter and the ORF25 polyadenylation sequence; the two are separated by a multiple cloning site. Thus, the strategy for constructing the vector was to move the yeast desaturase gene from pUC26A into pIC35A between the promoter and the termination sequences, and then to move it into the vector pH602.

A 1665 bp BclI-BspH1 fragment from pUC26A, which contained the genomic yeast desaturase coding sequence, was isolated; the BspH1 site was filled in and BamH1 linkers added. This fragment was then cloned into the BamH1 site of pIC35A; the resulting vector was designated pSOA. A 3075 XbaI fragment from pSOA, which contained the 35S promoter, the yeast desaturase gene coding sequence and the ORF25 3' polyadenylation sequence, was isolated; the fragment was blunt-ended with T4 DNA polymerase and cloned into the BglII site of pH602, which had been blunt-ended with T4 DNA polymerase. The resulting vector was designated pH.SOA.

EXAMPLE 3

Vector Transfer to Agrobacterium

All four plasmids described above, pH.PO, pH.POP, pH.PdeltaBOP, and pH.SOA, were moved into the Agrobacterium strain Z707s by tri-parental mating with the E. coli strains DH15 and RK2013 essentially as described (Rogers SG et al (1988) Plant Molecular Biology Manual A2 Kluwer Academic Publishers, Dordrecht), pp 1-12).

EXAMPLE 4

Rapese d transformation

Rapese d is one of the world's most important oilseed crops. Considerable effort has been made to improve its agronomic qualities by selective breeding techniques. Brassica napus and Brassica rapa constitute the majority of rapese d production in North America.

Brassica napus is fairly amenable to tissue culture, thus offering a good system for introduction of foreign genes. Transgenic plants of B. napus obtain d by Agrobacteria m diated transformation have been previously reported (Pua et al (1987) Bio/T chnology 5: 815-817; Fry et el (1987) Plant Cell Reports 6: 321-325; Radke et al (1988) Theor. Appl. Genet. 75: 685-694); and Moloney et al (1989) Plant Cell Reports 8: 238-242). Microinjection (Neuhaus et al (1987) Theor. Appl. Genet. 75: 30-36)) and protoplast electroporation (Guerche et al (1987) Plant Sci. 52: 111-116) techniques have also been used to transform B. napus. Brassica rapa may also be transformed, as was recently reported at by Mehra-Palta et al (1991, Proceedings of the Rapeseed GCIRC Congress, pp 1108-1115).

The plant used in this example of rapeseed transformation was the Brassica napus cultivar Profit. Seeds were obtained both from normal plants, and from plants obtained from a line of previously regenerated plants. This regenerate line of Profit results in plants whose tissue demonstrates an increased frequency of transformation, when the frequency is calculated as the number of transgenic plants obtained from a specified number of tissue explants. Seeds were surface sterilized with 1.05% sodium hypochlorite (20% Chlorox) for 20 minutes and rinsed 3 times with sterile distilled water. These seeds were aseptically germinated on basal medium(BM) in 20 x 100 mm petri dishes for 4 - 6 days. The BM consisted of Murashige and Skoog (1962) macro- and micro-elements, with iron at 40 mg/l FeNa2EDTA, and the following constituents (mg/l): myo-inositol, 100; nicotinic acid, 0.1; pyrodoxine HCl, 0.1; thiamine HCl, 0.02; glycine, 0.4; sucrose, 30,000; and Difco bacto agar, 8,000. The seedlings were grown at 25 oC with a photoperiod of 16 hours. Hypocotyl segments (2 -3 mm) were excised from 4 - 6 day old seedlings and pretreated for 24 hours on BM or Gamborgs' B5 (Gamborg et al, 1968) medium containing alpha-napthaleneacetic acid (NAA) at 5 mg/l or 2,4-dichlorophenoxyacetic acid (2,4-D) at 1 mg/l (callusing medium). A sterile filter paper was placed on the medium prior to treatment.

The hypocotyl segments were treated with the Agrobacterium solution (diluted to 10 * 8/ml with liquid basal medium) for 30 minutes and then placed onto the callusing medium for 2-3 days of co-cultivation.

The hypocotyl tissues were transferred to the callusing medium which contained carbenicillin (500 mg/l) and hygromycin (5-10 mg/l). The cultures were maintained at 22 +- 2 oC with a 16 hour photoperiod. After 7 days, the hypocotyl segments were transferred to shoot regeneration medium BM or B5, both of which contained BAP (1-4 mg/l), zeatin (0-4 mg/l), silver nitrate (AgNO3, 2.5-10 mg/l), carbenicillin (500 mg/l), and hygromycin (5-10 mg/l). The callusing and regeneration media were solidified with Agarose (SeaKem, 0.5%) or Gelrite (0.2%). The tissues were transferred to fresh selection medium every three weeks. Callus formation occurred after 1-3 weeks of culture, and shoots were formed 3-6 weeks thereafter. These shoots were then transferred to BM containing BAP (0.01-0.1 mg/l) and carbenicillin (100 mg/l) for elongation, and were later rooted on BM with indole butyric acid (IBA, 0.1 mg/l).

EXAMPLE 5

Determination of Plant Transformation

Each regenerated plant which survived on the selection medium was assayed to determine whether it was in fact transgenic by at least one of the following biological and molecular assays.

a. Leaf Disc Assay

The presence and expression of a gene may be determined by an assay of the activity of the protein which is encoded by the gene. The leaf disc assay is a biological assay which detects the activity of the selectable marker gene, HPT (which confers hygromycin resistance to the transformed tissue), by measuring tissue growth in the presence of hygromycin. Since both the HPT gene and the yeast desaturase gene are transferred together on a single piece of DNA, the presence of the HPT gene indicates that the yeast desaturase gene is also present in the tissue assayed, as separately confirmed by PCR analysis (see below).

Small leaf sections (2-3 mm square) obtained from shoots grown on selection medium were cultured on BM which contained BAP (4 mg/l), NAA (0.5 mg/l), and hygromycin (10 mg/l) for 3- 4 weeks. Those leaf sections which remained green, and produced callus, roots, or shoots, were determined to originate from transgenic plants. Nontransformed tissue (or "escapes") turned brown and died.

b. Polymerase Chain Reaction: DNA

The pres nce of a gene may be determined by assaying for the pres nce of its DNA in a tissue sample. Two such assay methods include a Polymeras Chain Reaction (PCR) assay and a Southern assay.

A Polymerase Chain Reaction (PCR) assay was utilized to analyze very small amounts of DNA for the pres-

ence of two genes, the yeast desaturase coding regions, and th HPT gene (which confers hygromycin resistance). Only 100 ng of DNA isolated from rapes ed leaf tissue was assayed per sample ssentially as describ d (in Current Protocols in Molecular Biology (1987) edited by Ausubel, RM t al; Greene Publishing Associates & Wiley-Interscience). Primers corresponding to positions +543 and +1277 in the coding sequence of the yeast desaturase gene resulted in the synthesis of a DNA fragment which appeared as a 751 bp band in those plants which contained the gene. In a similar fashion, primers to specific sections of th HPT gene resulted in the synthesis of a DNA fragment in those plants which contained the HPT gene.

c. Southern Analysis

A Southern analysis detects the presence of a specific sequence of DNA in a example by hybridization of a labelled probe to that sequence in the example DNA. Much more DNA is required for the analysis than is needed for a PCR assay (see above). The number of copies of the yeast desaturase gene transferred into transformed rapeseed plants can also be determined from Southern analysis.

10 ug of DNA per sample isolated from rapeseed tissue was digested with HindIII and subjected to Southern analysis essentially as described (Current Protocols in Molecular Biology (1987) edited by Ausubel, RM et al; Greene Publishing Associates & Wiley-Interscience). A 402 bp and 422 bp EcoRI doublet from the coding sequence of the yeast desaturase gene was labelled by the random hexamer procedure according to the manufacturer's instructions (United States Biochemical) and used as a probe.

EXAMPLE 6

Expression of Yeast delta-9 Desaturase in Seed Tissue

The expression of the yeast delta-9 desaturase in seed tissue results in transcription of the DNA to mRNA; the mRNA is in turn translated into the protein. Finally, the active protein desaturates saturated fatty acids. Thus, determination of expression of the yeast delta-9 desaturase gene in seed tissue may be determined by assaying for the presence of either mRNA, the protein, or an altered fatty acid composition in the seed oil.

a. Polymerase Chain Reaction: mRNA

Expression of the yeast desaturase gene is determined at the level of transcription by detecting the presence of the desaturase mRNA. This is accomplished by a linked reverse transcription and PCR assay modified from Frohman et al (1988; PNAS (USA) 85: 8998 - 9002) in which small amounts of tissue are analyzed for the presence of RNA transcripts from the desaturase gene.

b. Western Analysis

A Western analysis detects the presence of a protein by binding the protein, after separation by gel electrophoresis, to a labelled antibody. Thus, this method detects expression of a gene at the level of translation, or the protein level. It is preferable to assay the tissue when the highest level of expression of the gene is expected, for example, during oil accumulation during seed development.

Seeds from transgenic plants are collected at various times after pollination. Protein samples are prepared from pools of 10 seeds for each plant by homogenizing in SDS gel buffer (50mM Tris-HCl,pH 6.8, 1% SDS, 2 mM DTT and 2 mM EDTA). The homogenates are clarified by spinning in a microcentrifuge for 5 min. The proteins in the supernatant fractions are separated by 10% SDS-PAGE (Laemmli, UK (1970) Nature 227: 680-685), transferred to a nitrocellulose membrane (Towbin, H et al. (1979) Proc.Natl.Acd.Sci. USA 76: 4350-4354) and reacted sequentially with first rabbit polyclonal antiserum raised to a yeast desaturase peptide and then with anti-rabbit enzyme-conjugated (either alkaline phosphatase or horse radish peroxidase) IgG. The conjugated antibodies are visualized by activity staining according to the maunfacturer's protocol(s).

c. Fatty Acid Analysis

The fatty acid composition of rapeseed was determined as described below for either "half-seed" analysis, "single/whole seed" analysis, or "bulk se d" analyses (for example, the fatty acid methylation procedure is a modification of that reported Craig, BM and Murty, NL, 1959, J Amer Oil Chem Soc 36: 549 -552).

For "half-seed" analys s, a portion of cotyledonary tissue from the embryo was removed and analyzed; the remaining seed was th n saved, and could be germinated if desired.

1. The sample of cotyledonary tissu was placed into a 2 ml autosampl r vial.
2. n-Heptan (500 ul) was added, and th oil xtracted for 16 hours by incubation at room temperature.
3. Sodium methoxide in m thanol (50 ul of 0.5 M) was added, and the fatty acids transesterified for 60 minutes at room temperature.
4. Distilled water (20 ul) was then add d, and the vial capped with a TPFE lined crimp top cap. The sample was thus ready to be processed through the gas liquid chromatograph.

For "single-seed" analyses, a single seed was placed in a 2.0 ml autosampler vial and crushed with a glass rod.

1. n-Heptane (1.0 ml) was added, and the oil extracted for 16 hours by incubation at room temperature.
2. n-Heptane (3.0 ml) was added, and the oil extracted for one hour by incubation at room temperature.
3. Sodium methoxide in methanol (50 ul of 0.5N) was added, the vial vortexed, and the fatty acids transesterified for 60 minutes at room temperature.
4. Distilled water (20 ul) was added, the vial was vortexed and then capped with a TPFE crimp top cap. The sample was thus ready to be processed through the gas liquid chromatograph.

For "bulk-seed" analyses, six mature seeds were selected which were black in color and well filled.

1. The seeds were placed in a 16 x 100 mm disposable glass test tube.
2. n-Heptane (1.5 ml) was added, and the seeds ground with a tissue homogenizer. The oil was extracted for one minute.
3. Sodium methoxide in methanol (500 ul of 0.5N) was added, the tube vortexed, and the sample incubated for 5 minutes.
4. Distilled water (7.0 ml) was added, and the tube vortexed.
5. A portion of the organic layer (1.5 ml) was transferred to a 2.0 ml autosampler vial, and the vial then capped with a TPFE crimp top cap. The example was thus ready to be processed through the gas liquid chromatograph.

The GLC analyses were accomplished with a Hewlett Packard 5890 gas liquid chromatograph equipped with a flame ionization detector and a ChromStation integrator. A Hewlett Packard 7376 autosampler was used to withdraw a 1 ul portion of the methylated free fatty acids from the upper organic phase in the vial and to inject it into the GLC. The column used was a DB-23 fused silica capillary column (with a film thickness of 0.24 microns and column dimensions of 0.25 mm inner diameter x 30 mm long).

The operating conditions for the GLC analysis included an injector temperature of 250 oC and a detector temperature of 300 oC. The carrier gas was helium flowing at 1.1 cm3/minute through the column, and at 30 cm3/minute through the detector. Each chromatographic run began at 180 oC for 8 minutes; the temperature was then increased by 5 oC per minute to 220 oC, and then held at 220 oC for 4 minutes. With this program, all of the major fatty acid methyl esters expected for vegetable oils (ie, palmitate, stearate, oleate, linoleate, and linolenate) had eluted; in addition, the two isomers of the monounsaturated 18-carbon fatty acid methyl ester, oleate and cis-vaccenate, were separated from each other. The proportion of each fatty acid present was expressed as the percent by weight relative to the total fatty acid content of the seed.

EXAMPLE 7

Transformed Plants

The rapeseed B. napus Profit is a spring Canola-type rapeseed with a high oleate content in the seed oil. Analysis of fifty individual seeds results in the following fatty acid profile:

Table 1

a. Fatty Acid Profile of B. napus cv Profit

FATTY ACID	MEAN	MINIMUM	MAXIMUM
C16:0	4.05	3.20	7.40
C16:1	0.18	0.00	0.50
C18:0	2.07	1.20	3.80
C18:1D9	63.78	51.90	72.10
C18:1D11	2.72	1.80	5.90
C18:2	16.76	11.50	21.80
C18:3	6.82	3.60	11.20
C20:0	0.73	0.50	1.30
C20:1	1.24	0.90	1.50
C22:0	0.38	0.00	0.80
C24:0	0.24	0.00	0.90
C24:1	0.21	0.00	0.40

b. Fatty Acid Profile of regenerate B. napus cv Profit

FATTY ACID	MEAN	MINIMUM	MAXIMUM
C16:0	5.28	4.30	6.90
C16:1	0.26	0.10	0.50
C18:0	1.78	1.20	3.60
C18:1D9	51.51	40.70	63.70
C18:1D11	2.39	1.40	3.70
C18:2	25.35	16.30	35.00
C18:3	9.68	4.80	18.20
C20:0	0.66	0.40	1.30
C20:1	1.08	0.80	1.40
C22:0	0.40	0.20	0.90
C24:0	0.30	0.00	0.70
C24:1	0.29	0.00	0.70

Tissue obtained from Profit was transformed with each of four vectors as described above. The rooted transformed plants were transferred to soil when the shoots were 2 cm or more long. The plants were maintained in a Conviron growth chamber at 20 oC with 16 hours of light at 15 oC for 3-4 weeks; they were then moved into the greenhouse, where they were grown to maturity. Upon flowering, the plants were self-pollinated, and mature seed collected.

The fatty acid content of the resulting oil in the mature seeds is analyzed by either whole seed analysis, or by half-seed analysis in which a portion of the cotyledon is analyzed while the remaining seed is saved and can be planted.

Alternatively, in order to detect the presence of the yeast delta-9 desaturase protein in the seed, developing seeds are collected, and either mRNA is analyzed by a PCR assay or protein is assayed by a Western assay.

The fatty acid content of seeds obtained from rapeseed tissue transformed with the third vector, pH.PdeltaBOP, which was then regenerated and self-pollinated, contains a significant decrease in the proportions of the saturated fatty acids palmitate and stearate, with a concomitant increase in the levels of palmitoleate and oleate, when compared to the proportions observed in the non-transformed "parent" plant (see Table 1). In this vector, the yeast desaturase gene is placed under control of a modified phaseolin promoter. The modification, which consists of deleting the first 2/3 of the promoter, results in earlier expression of the regulated gene during seed development. Gene expression appears to occur during lipid accumulation, such that the unsaturated fatty acids are desaturated during triglycerol assembly. The resulting vegetable oil, with very low levels of saturated fatty acids, is a desirable substitute for vegetable oils currently on the market.

The fatty acid content of seeds obtained from plants transformed with any of the three other vectors, which are then regenerated and self-pollinated, contain varying proportions of the saturated fatty acids palmitate and stearate, which, however, are all equal to or lower than that observed in the non-transformed "parent" plant (see Table 1). These vectors, which contain regulatory elements which cause gene expression during seed development, result in variable levels of gene expression during lipid accumulation.

The seeds resulting from transformed, regenerated and self-pollinated plants are germinated and then self-

pollinated upon flowering. The resulting seeds are then analyzed to determine trait stability and gene inheritance.

EXAMPLE 8

Transfer of Yeast delta-Desaturase Gene into Other Brassica

The yeast delta-9 desaturase gene is transferred to other Brassica by one of two methods. One is to directly transform other rapeseed and oilseed mustard as described above, and the other is to move the trait into other rapeseed and oilseed mustard by classical breeding techniques. Brassica rapa, Brassica napus, and Brassica junceae are suitable plants for plant transformation (see above). In addition, they may be intermated in a breeding program.

This allows the transfer the yeast delta-9 desaturase to rapeseed and oilseed mustard selected on the basis of their initial fatty acid profiles or their agronomic characteristics. Some examples of rapeseed and oilseed mustard to which the yeast delta-9 desaturase gene is transferred are summarized in Table 2.

Table 2: Brassica cultivars to which yeast delta-nine desaturase gene is transferred

Brassica napus	
Spring Canola	Profit, Excel, Legend, Delta, proprietary high oleate/low linoleate strains
Spring HEAR	Hero
Winter Canola	Ceres, Tapidor, Samoris, proprietary high oleate and high oleate/low linoleate strains
Winter HEAR	Bridger, LEI
Brassica rapa	
Spring Canola	Parkland, Colt, Horizon, Svalof High palmitate, proprietary high oleate strains
Spring HEAR	R500
Brassica junceae	
Indian oilseed type High erucate	RH30, Puva Bold
Canadian oilseed type Low erucate	ZEM 87-1

The present invention has of necessity b en discussed herein by reference to certain specific methods and materials. The numeration of these methods and materials was merely illustrative, and in no way constitut s any limitation on the scope of the present invention. It is to be expected that those skilled in the art may

discern and practice variations of or alternatives to the specific teachings provided herein, without departing from the scope of the present invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: The Lubrizol Corporation
(B) STREET: 29400 Lakeland Boulevard
(C) CITY: Wickliffe
(D) STATE: Ohio
(E) COUNTRY: U.S.A.
(F) POSTAL CODE (ZIP): 44092

(ii) TITLE OF INVENTION: Modification of vegetable oils using desaturase

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 93301895.4

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1782 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae
(B) STRAIN: X2180-1A

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 106..1635

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TACTTCTGTT TCCGTTTATA TTTTGTATTA CGTAGAATAG AACATCATAG TAATAGATAG      60

TTGTGGTGAT CATATTATAA ACAGCACTAA AACATTACAA CAAAG ATG CCA ACT          114
                                                  Met Pro Thr
                                                   1

TCT GGA ACT ACT ATT GAA TTG ATT GAC GAC CAA TTT CCA AAG GAT GAC        162
Ser Gly Thr Thr Ile Glu Leu Ile Asp Asp Gln Phe Pro Lys Asp Asp
  5                  10                  15

TCT GCC AGC AGT GGC ATT GTC GAC GAA GTC GAC TTA ACG GAA GCT AAT        210
Ser Ala Ser Ser Gly Ile Val Asp Glu Val Asp Leu Thr Glu Ala Asn
 20                  25                  30                  35

ATT TTG GCT ACT GGT TTG AAT AAG AAA GCA CCA AGA ATT GTC AAC GGT        258
Ile Leu Ala Thr Gly Leu Asn Lys Lys Ala Pro Arg Ile Val Asn Gly
                 40                  45                  50

TTT GGT TCT TTA ATG GGC TCC AAG GAA ATG GTT TCC GTG GAA TTC GAC        306
Phe Gly Ser Leu Met Gly Ser Lys Glu Met Val Ser Val Glu Phe Asp
            55                  60                  65

AAG AAG GGA AAC GAA AAG AAG TCC AAT TTG GAT CGT CTG CTA GAA AAG        354
Lys Lys Gly Asn Glu Lys Lys Ser Asn Leu Asp Arg Leu Leu Glu Lys
        70                  75                  80

GAC AAC CAA GAA AAA GAA GAA GCT AAA ACT AAA ATT CAC ATC TCC GAA        402
Asp Asn Gln Glu Lys Glu Glu Ala Lys Thr Lys Ile His Ile Ser Glu
    85                  90                  95
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CAA CCA TGG ACT TTG AAT AAC TGG CAC CAA CAT TTG AAC TGG TTG AAC      450
Gln Pro Trp Thr Leu Asn Asn Trp His Gln His Leu Asn Trp Leu Asn
100                 105                 110                 115

ATG GTT CTT GTT TGT GGT ATG CCA ATG ATT GGT TGG TAC TTT GCT CTC      498
Met Val Leu Val Cys Gly Met Pro Met Ile Gly Trp Tyr Phe Ala Leu
                120                 125                 130

TCT GGT AAA GTG CCT TTG CAT TTA AAC GTT TTC CTT TTC TCC GTT TTC      546
Ser Gly Lys Val Pro Leu His Leu Asn Val Phe Leu Phe Ser Val Phe
            135                 140                 145

TAC TAC GCT GTC GGT GGT GTT TCT ATT ACT GCC GGT TAC CAT AGA TTA      594
Tyr Tyr Ala Val Gly Gly Val Ser Ile Thr Ala Gly Tyr His Arg Leu
        150                 155                 160

TGG TCT CAC AGA TCT TAC TCC GCT CAC TGG CCA TTG AGA TTA TTC TAC      642
Trp Ser His Arg Ser Tyr Ser Ala His Trp Pro Leu Arg Leu Phe Tyr
    165                 170                 175

GCT ATC TTC GGT TGT GCT TCC GTT GAA GGG TCC GCT AAA TGG TGG GGC      690
Ala Ile Phe Gly Cys Ala Ser Val Glu Gly Ser Ala Lys Trp Trp Gly
180                 185                 190                 195

CAC TCT CAC AGA ATT CAC CAT CGT TAC ACT GAT ACC TTG AGA GAT CCT      738
His Ser His Arg Ile His His Arg Tyr Thr Asp Thr Leu Arg Asp Pro
                200                 205                 210

TAT GAC GCT CGT AGA GGT CTA TGG TAC TCC CAC ATG GGA TGG ATG CTT      786
Tyr Asp Ala Arg Arg Gly Leu Trp Tyr Ser His Met Gly Trp Met Leu
            215                 220                 225

TTG AAG CCA AAT CCA AAA TAC AAG GCT AGA GCT GAT ATT ACC GAT ATG      834
Leu Lys Pro Asn Pro Lys Tyr Lys Ala Arg Ala Asp Ile Thr Asp Met
        230                 235                 240

ACT GAT GAT TGG ACC ATT AGA TTC CAA CAC AGA CAC TAC ATC TTG TTG      882
Thr Asp Asp Trp Thr Ile Arg Phe Gln His Arg His Tyr Ile Leu Leu
    245                 250                 255

ATG TTG TTA ACC GCT TTC GTC ATT CCA ACT CTT ATC TGT GGT TAC TTT      930
Met Leu Leu Thr Ala Phe Val Ile Pro Thr Leu Ile Cys Gly Tyr Phe
260                 265                 270                 275

TTC AAC GAC TAT ATG GGT GGT TTG ATC TAT GCC GGT TTT ATT CGT GTC      978
Phe Asn Asp Tyr Met Gly Gly Leu Ile Tyr Ala Gly Phe Ile Arg Val
                280                 285                 290

TTT GTC ATT CAA CAA GCT ACC TTT TGC ATT AAC TCC TTG GCT CAT TAC     1026
Phe Val Ile Gln Gln Ala Thr Phe Cys Ile Asn Ser Leu Ala His Tyr
            295                 300                 305
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ATC GGT ACC CAA CCA TTC GAT GAC AGA AGA ACC CCT CGT GAC AAC TGG      1074
Ile Gly Thr Gln Pro Phe Asp Asp Arg Arg Thr Pro Arg Asp Asn Trp
        310                 315                 320

ATT ACT GCC ATT GTT ACT TTC GGT GAA GGT TAC CAT AAC TTC CAC CAC      1122
Ile Thr Ala Ile Val Thr Phe Gly Glu Gly Tyr His Asn Phe His His
    325                 330                 335

GAA TTC CCA ACT GAT TAC AGA AAC GCT ATT AAG TGG TAC CAA TAC GAC      1170
Glu Phe Pro Thr Asp Tyr Arg Asn Ala Ile Lys Trp Tyr Gln Tyr Asp
340                 345                 350                 355

CCA ACT AAG GTT ATC ATC TAT TTG ACT TCT TTA GTT GGT CTA GCA TAC      1218
Pro Thr Lys Val Ile Ile Tyr Leu Thr Ser Leu Val Gly Leu Ala Tyr
                360                 365                 370

GAC TTG AAG AAA TTC TCT CAA AAT GCT ATT GAA GAA GCC TTG ATT CAA      1266
Asp Leu Lys Lys Phe Ser Gln Asn Ala Ile Glu Glu Ala Leu Ile Gln
            375                 380                 385

CAA GAA CAA AAG AAG ATC AAT AAA AAG AAG GCT AAG ATT AAC TGG GGT      1314
Gln Glu Gln Lys Lys Ile Asn Lys Lys Lys Ala Lys Ile Asn Trp Gly
        390                 395                 400

CCA GTT TTG ACT GAT TTG CCA ATG TGG GAC AAA CAA ACC TTC TTG GCT      1362
Pro Val Leu Thr Asp Leu Pro Met Trp Asp Lys Gln Thr Phe Leu Ala
    405                 410                 415

AAG TCT AAG GAA AAC AAG GGT TTG GTT ATC ATT TCT GGT ATT GTT CAC      1410
Lys Ser Lys Glu Asn Lys Gly Leu Val Ile Ile Ser Gly Ile Val His
420                 425                 430                 435

GAC GTA TCT GGT TAT ATC TCT GAA CAT CCA GGT GGT GAA ACT TTA ATT      1458
Asp Val Ser Gly Tyr Ile Ser Glu His Pro Gly Gly Glu Thr Leu Ile
                440                 445                 450

AAA ACT GCA TTA GGT AAG GAC GCT ACC AAG GCT TTC AGT GGT GGT GTC      1506
Lys Thr Ala Leu Gly Lys Asp Ala Thr Lys Ala Phe Ser Gly Gly Val
            455                 460                 465

TAC CGT CAC TCA AAT GCC GCT CAA AAT GTC TTG GCT GAT ATG AGA GTG      1554
Tyr Arg His Ser Asn Ala Ala Gln Asn Val Leu Ala Asp Met Arg Val
        470                 475                 480

GCT GTT ATC AAG GAA AGT AAG AAC TCT GCT ATT AGA ATG GCT AGT AAG      1602
Ala Val Ile Lys Glu Ser Lys Asn Ser Ala Ile Arg Met Ala Ser Lys
    485                 490                 495

AGA GGT GAA ATC TAC GAA ACT GGT AAG TTC TTT TAAGTATCAC ATTACAATAA    1655
Arg Gly Glu Ile Tyr Glu Thr Gly Lys Phe Phe
500                 505                 510
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CAAAACTGCA ACTACCATAA AAAAAAATTG AAAAATCATA AATTAAAAAA AAAAAAATCA 1715

ATTGAATTTT TTTTTTTCAT GATTACGTTT TGACATTTTT TCTTTTTTTT TCTCTTATTA 1775

CGATTTA 1782

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 510 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Pro Thr Ser Gly Thr Thr Ile Glu Leu Ile Asp Asp Gln Phe Pro
 1               5                  10                  15

Lys Asp Asp Ser Ala Ser Ser Gly Ile Val Asp Glu Val Asp Leu Thr
            20                  25                  30

Glu Ala Asn Ile Leu Ala Thr Gly Leu Asn Lys Lys Ala Pro Arg Ile
        35                  40                  45

Val Asn Gly Phe Gly Ser Leu Met Gly Ser Lys Glu Met Val Ser Val
    50                  55                  60

Glu Phe Asp Lys Lys Gly Asn Glu Lys Lys Ser Asn Leu Asp Arg Leu
65                  70                  75                  80

Leu Glu Lys Asp Asn Gln Glu Lys Glu Glu Ala Lys Thr Lys Ile His
                85                  90                  95

Ile Ser Glu Gln Pro Trp Thr Leu Asn Asn Trp His Gln His Leu Asn
            100                 105                 110

Trp Leu Asn Met Val Leu Val Cys Gly Met Pro Met Ile Gly Trp Tyr
        115                 120                 125

Phe Ala Leu Ser Gly Lys Val Pro Leu His Leu Asn Val Phe Leu Phe
    130                 135                 140

Ser Val Phe Tyr Tyr Ala Val Gly Gly Val Ser Ile Thr Ala Gly Tyr
145                 150                 155                 160

His Arg Leu Trp Ser His Arg Ser Tyr Ser Ala His Trp Pro Leu Arg
                165                 170                 175

Leu Phe Tyr Ala Ile Phe Gly Cys Ala Ser Val Glu Gly Ser Ala Lys
            180                 185                 190

Trp Trp Gly His Ser His Arg Ile His His Arg Tyr Thr Asp Thr Leu
        195                 200                 205

Arg Asp Pro Tyr Asp Ala Arg Arg Gly Leu Trp Tyr Ser His Met Gly
    210                 215                 220
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Trp Met Leu Leu Lys Pro Asn Pro Lys Tyr Lys Ala Arg Ala Asp Ile
225                 230                 235                 240

Thr Asp Met Thr Asp Asp Trp Thr Ile Arg Phe Gln His Arg His Tyr
                245                 250                 255

Ile Leu Leu Met Leu Leu Thr Ala Phe Val Ile Pro Thr Leu Ile Cys
            260                 265                 270

Gly Tyr Phe Phe Asn Asp Tyr Met Gly Gly Leu Ile Tyr Ala Gly Phe
        275                 280                 285

Ile Arg Val Phe Val Ile Gln Gln Ala Thr Phe Cys Ile Asn Ser Leu
    290                 295                 300

Ala His Tyr Ile Gly Thr Gln Pro Phe Asp Asp Arg Arg Thr Pro Arg
305                 310                 315                 320

Asp Asn Trp Ile Thr Ala Ile Val Thr Phe Gly Glu Gly Tyr His Asn
                325                 330                 335

Phe His His Glu Phe Pro Thr Asp Tyr Arg Asn Ala Ile Lys Trp Tyr
            340                 345                 350

Gln Tyr Asp Pro Thr Lys Val Ile Ile Tyr Leu Thr Ser Leu Val Gly
        355                 360                 365

Leu Ala Tyr Asp Leu Lys Lys Phe Ser Gln Asn Ala Ile Glu Glu Ala
    370                 375                 380

Leu Ile Gln Gln Glu Gln Lys Lys Ile Asn Lys Lys Lys Ala Lys Ile
385                 390                 395                 400

Asn Trp Gly Pro Val Leu Thr Asp Leu Pro Met Trp Asp Lys Gln Thr
                405                 410                 415

Phe Leu Ala Lys Ser Lys Glu Asn Lys Gly Leu Val Ile Ile Ser Gly
            420                 425                 430

Ile Val His Asp Val Ser Gly Tyr Ile Ser Glu His Pro Gly Gly Glu
        435                 440                 445

Thr Leu Ile Lys Thr Ala Leu Gly Lys Asp Ala Thr Lys Ala Phe Ser
    450                 455                 460

Gly Gly Val Tyr Arg His Ser Asn Ala Ala Gln Asn Val Leu Ala Asp
465                 470                 475                 480

Met Arg Val Ala Val Ile Lys Glu Ser Lys Asn Ser Ala Ile Arg Met
                485                 490                 495

Ala Ser Lys Arg Gly Glu Ile Tyr Glu Thr Gly Lys Phe Phe
            500                 505                 510
```

Claims

1. A plant seed comprising a y ast delta-9 desaturase gene and means for expressing said yeast delta-9 desaturase gene in said plant seed.

2. The plant se d as defined by claim 1, wherein said means for expressing comprises a promoter effective to cause xpression of said yeast delta-9 desaturas gene in said plant se d.

3. The plat seed as defined by claim 2, wherein said promoter is selected from a phaseolin promoter, a truncated phaseolin promoter, and a 35S promoter.

4. The plant seed as defined by either of claims 2 and 3, wherein said promoter is a seed-specific promoter.

5. The plant seed as defined by any one of claims 1 to 4, further comprising a termination sequence for said yeast delta-9 desaturase gene.

6. The plant seed as defined by claim 5, wherein said termination sequence is selected from a yeast delta-9 desaturase termination sequence, a phaseolin 3' termination sequence, and a ORF 25 3' termination sequence.

7. The plant seed as defined by claim 6, wherein said termination sequence is an ORF 25 termination sequence.

8. The plant seed as defined by any one of claims 1 to 7, wherein said plant seed is a seed of a monocot plant genus.

9. The plant seed as defined by claim 8, wherein said monocot plant genus is selected from Zea and Sorghum.

10. The plant seed as defined by either of claims 8 and 9, wherein said monocot plant genus is Zea.

11. The plant seed as defined by claim 10, further wherein said plant seed is a seed of Zea maize.

12. The plant seed as defined by any one of claims 1 to 7, wherein said plant seed is a seed of a dicot plant genus.

13. The plant seed as defined by claim 12, wherein said dicot plant genus is selected from Brassica, Helianthus, Carthamus, Sesamum, Glycine, Arachis, Gossypium, Lesquerella, and Vernonia.

14. The plant seed as defined by claim 13, wherein said dicot plant genus is Brassica.

15. The plant seed as defined by claim 14, further wherein said plant seed is a seed selected from Brassica rapa and Brassica napus.

16. A method for modifying the fatty acid content of the seed oil of a plant seed, said method comprising the step of transforming the plant seed to express a yeast delta-9 desaturase gene.

17. The method as defined by claim 16, wherein said modifying comprises increasing the percent content of monounsaturated fatty acid in the seed oil of said plant seed.

18. The method as defined by claim 17, wherein said monounsaturated fatty acid has a carbon chain length of from 16 to 24 carbon atoms.

19. The method as defined by claim 18, wherein said monounsaturated fatty acid is selected from cis-9-hexadecanoic acid (palmitoleic acid), cis-9-octadecanoic acid (oleic acid), cis-11-octadecenoic acid (cis-vaccenic acid), cis-11-eicosenoic acid, cis-13-eicosenoic acid, cis-13-docosenoic acid, cis-15-docosenoic acid, cis-15-tetracosenoic acid, cis-17-tetracosenoic acid, and combinations thereof.

20. The method as defined by claim 19, wherein said monounsaturated fatty acid is oleic acid.

21. The method as defin d by claim 19, wherein said monounsaturated fatty acid is palmitoleic acid.

22. The method as defined by claim 19, wherein said monounsaturated fatty acid is cis vaccenic acid.

23. The method as defined by any one of claims 16 to 22 wh rein said modifying comprises r ducing the percent content of saturated fatty acid in the seed oil of said plant seed.

24. The method as defined by claim 23, wherein said saturated fatty acid is s lected from myristic acid, palmitic acid, stearic acid, eicosanoic acid, docosanoic acid, tetracosanoic acid, and combinations thereof.

25. The method as defined by any one of claims 16 to 24, wherein said plant seed is a seed of a monocot plant genus.

26. The method as defined by claim 25, wherein said monocot plant genus is selected from Zea and Sorghum.

27. The method as defined by claim 26, wherein said monocot plant genus is Zea.

28. The method as defined by claim 27, further wherein said plant seed is a seed of Zea maize.

29. The method as defined by any one of claims 16 to 24, wherein said plant seed is a seed of a dicot plant genus.

30. The method as defined by claim 29, wherein said dicot plant genus is selected from Brassica, Helianthus, Carthamus, Sesamum, Glycine, Arachis, Gossypium, Lesquerella, and Vernonia.

31. The method as defined by claim 30, wherein said dicot plant genus is Brassica.

32. The method as defined by claim 31, further wherein said plant seed is a seed selected from Brassica rapa and Brassica napus.

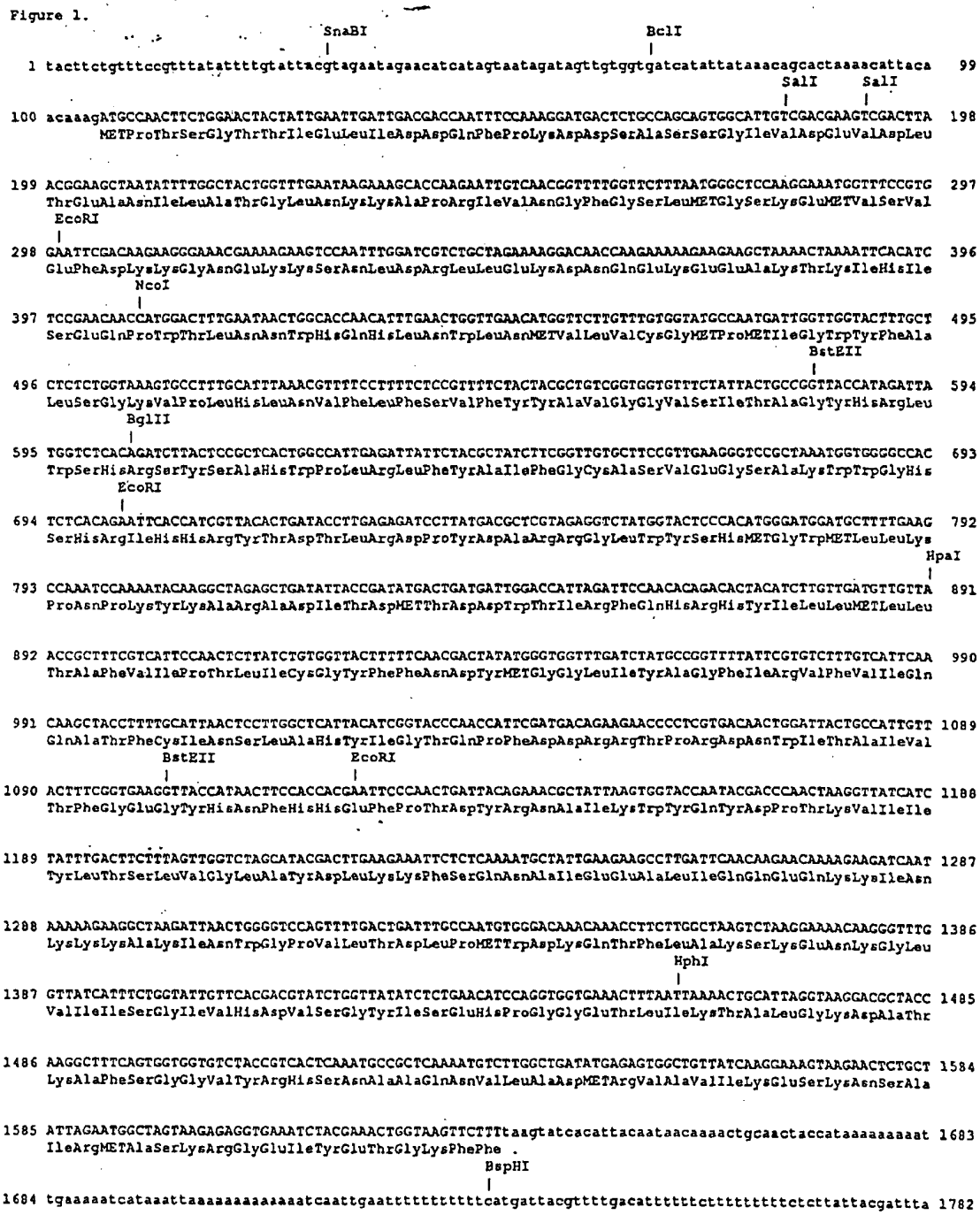
33. The method as defined by any one of claims 16 to 32, wherein said step of transforming comprises adding, to the native DNA of said plant seed, exogenous DNA, said exogenous DNA comprising yeast delta-9 desaturase gene and a promoter for said yeast delta-9 desaturase gene.

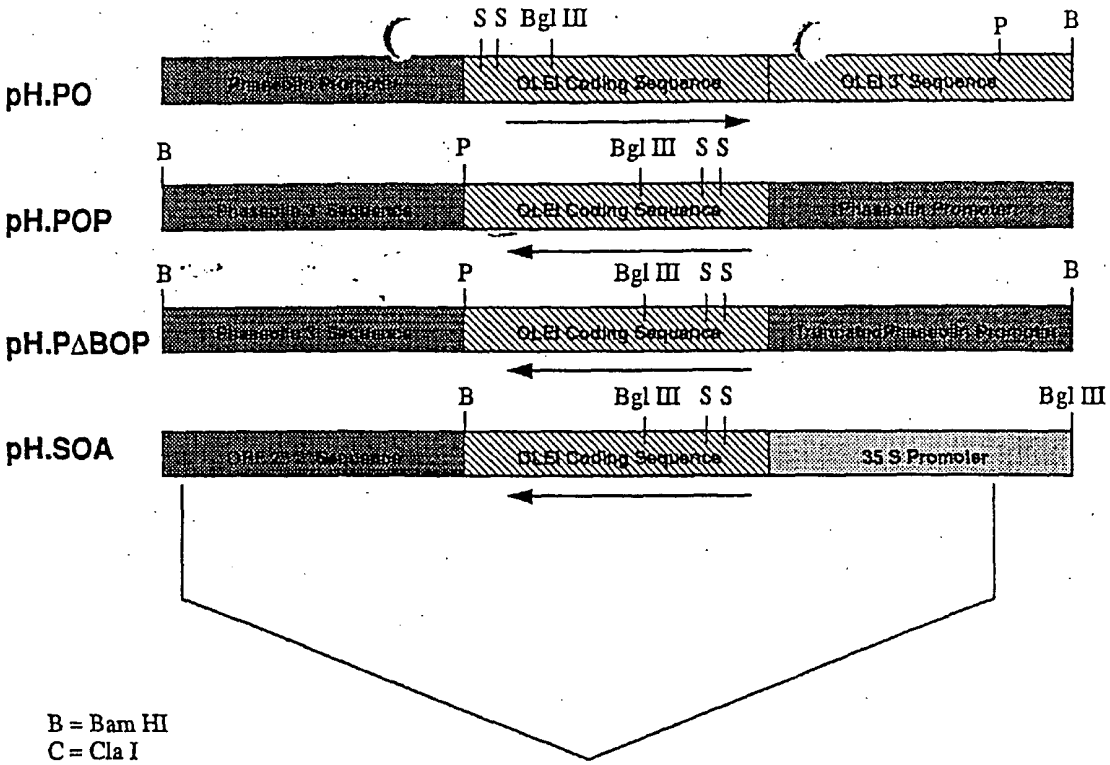
34. The method as defined by claim 33, wherein said step of transforming is carried out using transformation mediation selected from Agrobacterium, electroporation, polyethylene glycol (PEG), silicon carbide fiber, particle gun, and direct injection.

35. The method as defined by either of claims 33 and 34, further comprising constructing a vector containing said yeast delta-9 desaturase gene and said promoter, placing said vector into a selected strain of Agrobacterium, and treating selected plant cells with said Abrobacterium under conditions sufficient to result in transfer of at least some of said vectors from said Agrobacterium to said plant cells, whereby said yeast delta-9 desaturase gene is expressed in said plant cells.

36. A plant obtained from the plant seed as defined by any one of claims 1 to 15, wherein the seeds of said plant comprise a yeast delta-9 desaturase gene and means for expressing said yeast delta-9 desaturase gene in said plant seed.

Figure 1.


SnaBI
BclI
SalI
SalI
EcoRI
NcoI
BstEII
BglII
EcoRI
HpaI
BstEII
EcoRI
HphI
BspHI



B = Bam HI
C = Cla I
H = Hind III
K = Kpn I
P = Pst I
Pu = PVU I
RI = Eco RI
R5 = Eco RV
Sa = Sal I
Xh = Xho I

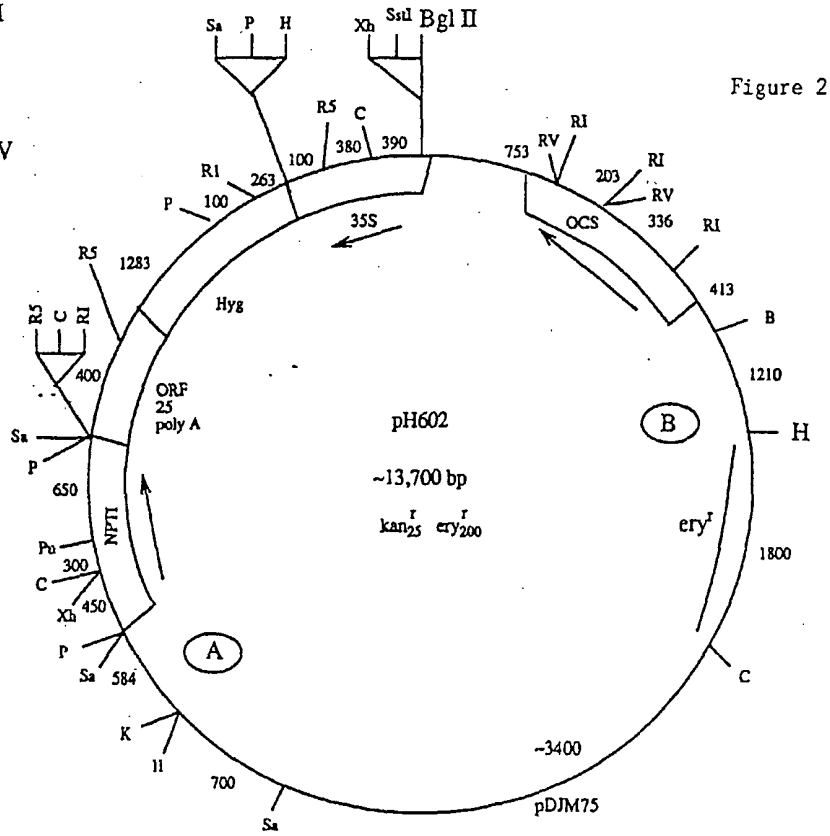


Figure 2

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1782 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae
(B) STRAIN: X2180-1A

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 106..1635

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TACTTCTGTT TCCGTTTATA TTTTGTATTA CGTAGAATAG AACATCATAG TAATAGATAG      60

TTGTGGTGAT CATATTATAA ACAGCACTAA AACATTACAA CAAAG ATG CCA ACT          114
                                                  Met Pro Thr
                                                    1

TCT GGA ACT ACT ATT GAA TTG ATT GAC GAC CAA TTT CCA AAG GAT GAC        162
Ser Gly Thr Thr Ile Glu Leu Ile Asp Asp Gln Phe Pro Lys Asp Asp
  5                  10                  15

TCT GCC AGC AGT GGC ATT GTC GAC GAA GTC GAC TTA ACG GAA GCT AAT        210
Ser Ala Ser Ser Gly Ile Val Asp Glu Val Asp Leu Thr Glu Ala Asn
 20                  25                  30                  35

ATT TTG GCT ACT GGT TTG AAT AAG AAA GCA CCA AGA ATT GTC AAC GGT        258
Ile Leu Ala Thr Gly Leu Asn Lys Lys Ala Pro Arg Ile Val Asn Gly
                 40                  45                  50

TTT GGT TCT TTA ATG GGC TCC AAG GAA ATG GTT TCC GTG GAA TTC GAC        306
Phe Gly Ser Leu Met Gly Ser Lys Glu Met Val Ser Val Glu Phe Asp
             55                  60                  65

AAG AAG GGA AAC GAA AAG AAG TCC AAT TTG GAT CGT CTG CTA GAA AAG        354
Lys Lys Gly Asn Glu Lys Lys Ser Asn Leu Asp Arg Leu Leu Glu Lys
         70                  75                  80

GAC AAC CAA GAA AAA GAA GAA GCT AAA ACT AAA ATT CAC ATC TCC GAA        402
Asp Asn Gln Glu Lys Glu Glu Ala Lys Thr Lys Ile His Ile Ser Glu
 85                  90                  95

CAA CCA TGG ACT TTG AAT AAC TGG CAC CAA CAT TTG AAC TGG TTG AAC        450
Gln Pro Trp Thr Leu Asn Asn Trp His Gln His Leu Asn Trp Leu Asn
100                 105                 110                 115

ATG GTT CTT GTT TGT GGT ATG CCA ATG ATT GGT TGG TAC TTT GCT CTC        498
Met Val Leu Val Cys Gly Met Pro Met Ile Gly Trp Tyr Phe Ala Leu
                120                 125                 130

TCT GGT AAA GTG CCT TTG CAT TTA AAC GTT TTC CTT TTC TCC GTT TTC        546
Ser Gly Lys Val Pro Leu His Leu Asn Val Phe Leu Phe Ser Val Phe
            135                 140                 145

TAC TAC GCT GTC GGT GGT GTT TCT ATT ACT GCC GGT TAC CAT AGA TTA        594
Tyr Tyr Ala Val Gly Gly Val Ser Ile Thr Ala Gly Tyr His Arg Leu
        150                 155                 160

TGG TCT CAC AGA TCT TAC TCC GCT CAC TGG CCA TTG AGA TTA TTC TAC        642
Trp Ser His Arg Ser Tyr Ser Ala His Trp Pro Leu Arg Leu Phe Tyr
    165                 170                 175
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GCT ATC TTC GGT TGT GCT TCC GTT GAA GGG TCC GCT AAA TGG TGG GGC          690
Ala Ile Phe Gly Cys Ala Ser Val Glu Gly Ser Ala Lys Trp Trp Gly
180                 185                 190                 195

CAC TCT CAC AGA ATT CAC CAT CGT TAC ACT GAT ACC TTG AGA GAT CCT          738
His Ser His Arg Ile His His Arg Tyr Thr Asp Thr Leu Arg Asp Pro
                200                 205                 210

TAT GAC GCT CGT AGA GGT CTA TGG TAC TCC CAC ATG GGA TGG ATG CTT          786
Tyr Asp Ala Arg Arg Gly Leu Trp Tyr Ser His Met Gly Trp Met Leu
            215                 220                 225

TTG AAG CCA AAT CCA AAA TAC AAG GCT AGA GCT GAT ATT ACC GAT ATG          834
Leu Lys Pro Asn Pro Lys Tyr Lys Ala Arg Ala Asp Ile Thr Asp Met
        230                 235                 240

ACT GAT GAT TGG ACC ATT AGA TTC CAA CAC AGA CAC TAC ATC TTG TTG          882
Thr Asp Asp Trp Thr Ile Arg Phe Gln His Arg His Tyr Ile Leu Leu
    245                 250                 255

ATG TTG TTA ACC GCT TTC GTC ATT CCA ACT CTT ATC TGT GGT TAC TTT          930
Met Leu Leu Thr Ala Phe Val Ile Pro Thr Leu Ile Cys Gly Tyr Phe
260                 265                 270                 275

TTC AAC GAC TAT ATG GGT GGT TTG ATC TAT GCC GGT TTT ATT CGT GTC          978
Phe Asn Asp Tyr Met Gly Gly Leu Ile Tyr Ala Gly Phe Ile Arg Val
                280                 285                 290

TTT GTC ATT CAA CAA GCT ACC TTT TGC ATT AAC TCC TTG GCT CAT TAC          1026
Phe Val Ile Gln Gln Ala Thr Phe Cys Ile Asn Ser Leu Ala His Tyr
            295                 300                 305

ATC GGT ACC CAA CCA TTC GAT GAC AGA AGA ACC CCT CGT GAC AAC TGG          1074
Ile Gly Thr Gln Pro Phe Asp Asp Arg Arg Thr Pro Arg Asp Asn Trp
        310                 315                 320

ATT ACT GCC ATT GTT ACT TTC GGT GAA GGT TAC CAT AAC TTC CAC CAC          1122
Ile Thr Ala Ile Val Thr Phe Gly Glu Gly Tyr His Asn Phe His His
    325                 330                 335

GAA TTC CCA ACT GAT TAC AGA AAC GCT ATT AAG TGG TAC CAA TAC GAC          1170
Glu Phe Pro Thr Asp Tyr Arg Asn Ala Ile Lys Trp Tyr Gln Tyr Asp
340                 345                 350                 355

CCA ACT AAG GTT ATC ATC TAT TTG ACT TCT TTA GTT GGT CTA GCA TAC          1218
Pro Thr Lys Val Ile Ile Tyr Leu Thr Ser Leu Val Gly Leu Ala Tyr
                360                 365                 370

GAC TTG AAG AAA TTC TCT CAA AAT GCT ATT GAA GAA GCC TTG ATT CAA          1266
Asp Leu Lys Lys Phe Ser Gln Asn Ala Ile Glu Glu Ala Leu Ile Gln
            375                 380                 385

CAA GAA CAA AAG AAG ATC AAT AAA AAG AAG GCT AAG ATT AAC TGG GGT          1314
Gln Glu Gln Lys Lys Ile Asn Lys Lys Lys Ala Lys Ile Asn Trp Gly
        390                 395                 400
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CCA GTT TTG ACT GAT TTG CCA ATG TGG GAC AAA CAA ACC TTC TTG GCT      1362
Pro Val Leu Thr Asp Leu Pro Met Trp Asp Lys Gln Thr Phe Leu Ala
    405                 410                 415

AAG TCT AAG GAA AAC AAG GGT TTG GTT ATC ATT TCT GGT ATT GTT CAC      1410
Lys Ser Lys Glu Asn Lys Gly Leu Val Ile Ile Ser Gly Ile Val His
420                 425                 430                 435

GAC GTA TCT GGT TAT ATC TCT GAA CAT CCA GGT GGT GAA ACT TTA ATT      1458
Asp Val Ser Gly Tyr Ile Ser Glu His Pro Gly Gly Glu Thr Leu Ile
                440                 445                 450

AAA ACT GCA TTA GGT AAG GAC GCT ACC AAG GCT TTC AGT GGT GGT GTC      1506
Lys Thr Ala Leu Gly Lys Asp Ala Thr Lys Ala Phe Ser Gly Gly Val
            455                 460                 465

TAC CGT CAC TCA AAT GCC GCT CAA AAT GTC TTG GCT GAT ATG AGA GTG      1554
Tyr Arg His Ser Asn Ala Ala Gln Asn Val Leu Ala Asp Met Arg Val
        470                 475                 480

GCT GTT ATC AAG GAA AGT AAG AAC TCT GCT ATT AGA ATG GCT AGT AAG      1602
Ala Val Ile Lys Glu Ser Lys Asn Ser Ala Ile Arg Met Ala Ser Lys
    485                 490                 495

AGA GGT GAA ATC TAC GAA ACT GGT AAG TTC TTT TAAGTATCAC ATTACAATAA    1655
Arg Gly Glu Ile Tyr Glu Thr Gly Lys Phe Phe
500                 505                 510

CAAAACTGCA ACTACCATAA AAAAAAATTG AAAAATCATA AATTAAAAAA AAAAAAATCA    1715

ATTGAATTTT TTTTTTTCAT GATTACGTTT TGACATTTTT TCTTTTTTTT TCTCTTATTA    1775

CGATTTA                                                              1782
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INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 510 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Pro Thr Ser Gly Thr Thr Ile Glu Leu Ile Asp Asp Gln Phe Pro
 1               5                  10                  15

Lys Asp Asp Ser Ala Ser Ser Gly Ile Val Asp Glu Val Asp Leu Thr
            20                  25                  30

Glu Ala Asn Ile Leu Ala Thr Gly Leu Asn Lys Lys Ala Pro Arg Ile
        35                  40                  45
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Val Asn Gly Phe Gly Ser Leu Met Gly Ser Lys Glu Met Val Ser Val
     50                  55                  60

Glu Phe Asp Lys Lys Gly Asn Glu Lys Lys Ser Asn Leu Asp Arg Leu
 65                  70                  75                  80

Leu Glu Lys Asp Asn Gln Glu Lys Glu Glu Ala Lys Thr Lys Ile His
                 85                  90                  95

Ile Ser Glu Gln Pro Trp Thr Leu Asn Asn Trp His Gln His Leu Asn
            100                 105                 110

Trp Leu Asn Met Val Leu Val Cys Gly Met Pro Met Ile Gly Trp Tyr
        115                 120                 125

Phe Ala Leu Ser Gly Lys Val Pro Leu His Leu Asn Val Phe Leu Phe
    130                 135                 140

Ser Val Phe Tyr Tyr Ala Val Gly Gly Val Ser Ile Thr Ala Gly Tyr
145                 150                 155                 160

His Arg Leu Trp Ser His Arg Ser Tyr Ser Ala His Trp Pro Leu Arg
                165                 170                 175

Leu Phe Tyr Ala Ile Phe Gly Cys Ala Ser Val Glu Gly Ser Ala Lys
            180                 185                 190

Trp Trp Gly His Ser His Arg Ile His His Arg Tyr Thr Asp Thr Leu
        195                 200                 205

Arg Asp Pro Tyr Asp Ala Arg Arg Gly Leu Trp Tyr Ser His Met Gly
    210                 215                 220

Trp Met Leu Leu Lys Pro Asn Pro Lys Tyr Lys Ala Arg Ala Asp Ile
225                 230                 235                 240

Thr Asp Met Thr Asp Asp Trp Thr Ile Arg Phe Gln His Arg His Tyr
                245                 250                 255

Ile Leu Leu Met Leu Leu Thr Ala Phe Val Ile Pro Thr Leu Ile Cys
            260                 265                 270

Gly Tyr Phe Phe Asn Asp Tyr Met Gly Gly Leu Ile Tyr Ala Gly Phe
        275                 280                 285

Ile Arg Val Phe Val Ile Gln Gln Ala Thr Phe Cys Ile Asn Ser Leu
    290                 295                 300

Ala His Tyr Ile Gly Thr Gln Pro Phe Asp Asp Arg Arg Thr Pro Arg
305                 310                 315                 320

Asp Asn Trp Ile Thr Ala Ile Val Thr Phe Gly Glu Gly Tyr His Asn
                325                 330                 335

Phe His His Glu Phe Pro Thr Asp Tyr Arg Asn Ala Ile Lys Trp Tyr
            340                 345                 350
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Gln Tyr Asp Pro Thr Lys Val Ile Ile Tyr Leu Thr Ser Leu Val Gly
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370 375 380

Leu Ile Gln Gln Glu Gln Lys Lys Ile Asn Lys Lys Lys Ala Lys Ile
385 390 395 400

Asn Trp Gly Pro Val Leu Thr Asp Leu Pro Met Trp Asp Lys Gln Thr
405 410 415

Phe Leu Ala Lys Ser Lys Glu Asn Lys Gly Leu Val Ile Ile Ser Gly
420 425 430

Ile Val His Asp Val Ser Gly Tyr Ile Ser Glu His Pro Gly Gly Glu
435 440 445

Thr Leu Ile Lys Thr Ala Leu Gly Lys Asp Ala Thr Lys Ala Phe Ser
450 455 460

Gly Gly Val Tyr Arg His Ser Asn Ala Ala Gln Asn Val Leu Ala Asp
465 470 475 480

Met Arg Val Ala Val Ile Lys Glu Ser Lys Asn Ser Ala Ile Arg Met
485 490 495

Ala Ser Lys Arg Gly Glu Ile Tyr Glu Thr Gly Lys Phe Phe
500 505 510